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ORIGINAL ARTICLE

Priming with r-metHuSCF and filgrastim or chemotherapy and filgrastim in patients with malignant lymphomas: a randomized phase II pilot study of mobilization and engraftment

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SCF has been shown to synergize with G-CSF to mobilize CD34$^+$ PBPCs. In this study we report results from this combination after a phase II trial of 32 patients with malignant lymphoma randomized to receive recombinant methionyl human SCF (ancestim, r-metHuSCF) in combination with recombinant methionyl human G-CSF (filgrastim, r-metHuG-CSF) (experimental arm A) or routine chemotherapy plus filgrastim (conventional arm B). The primary objective was to evaluate the side effects and toxicity during priming and mobilization. The secondary objectives were efficacy by the level of blood-circulating PBPCs, the number of harvest days and the time to three-lineage engraftment after autografting. First, during priming 5 patients had 8 serious events, 4 in each arm. A summary of all adverse events revealed 30 (94%) patients suffering from 132 events of all grading. Second, neutropenia and thrombocytopenia was documented in arm B. Third, 9/14 (64%) patients in arm A reached the target of 5 million CD34$^+$ cells/kg body weight (bw) compared with 13/15 (87%) in arm B. The results represent the first randomized trial of growth factor plus chemotherapy priming and indicate that a formal phase III trial very unlikely may challenge chemotherapy plus r-metHuG-CSF priming in candidates for high-dose therapy.
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Introduction

Auto-SCT is used to support high-dose chemotherapy in hematological malignancies.[1–2] PBPCs have replaced BM cells as the preferred source for transplantation because of faster blood cell recovery.[3–4] One variable of major effect for post transplant care is the number of PBPCs harvested.[5–8] Therefore, several clinical studies have aimed to identify priming regimens that improve progenitor and stem cell mobilizations and collections without increased toxicity. Frequently, filgrastim (recombinant methionyl human G-CSF (r-metHuG-CSF)) is administrated alone; however, filgrastim combined with chemotherapy has proven more effective in the context of CD34$^+$ cell numbers harvested,[9–11] and this combination is considered the gold standard for priming and stem cell mobilization in relapsed malignant lymphoma.

SCF is a glycoprotein growth factor that exerts an effect on hematopoietic blood cell progenitors.[12] Although SCF alone exerts little colony-stimulating activity on normal human BM cells *in vitro*, a combination of SCF with other recombinant hematopoietic cytokines results in a synergistic increase in the numbers of colonies.[13] *In vivo*, the addition of SCF to G-CSF (filgrastim) synergistically increases PBPC mobilization compared with filgrastim alone.[14–17] Several clinical trials have reported the ability of the combination of SCF with filgrastim to mobilize PBPCs in patients with lymphoma, multiple myeloma, breast and ovarian cancers even in heavily pretreated patients.[18–26]

Priming using chemotherapy is toxic and costly,[11] and new priming procedures need to be established, which is the background for this randomized pilot study. The hypothesis is that elimination of chemotherapy from the priming regimen may decrease the overall toxicity and the ability to collect a sufficient autograft, which, however, may be circumvented by adding r-metHuSCF (ancestim) to the priming regimen. The aim of this randomized phase II trial was to evaluate the safety, toxicity and efficacy of growth factors in lymphoma patients who were considered candidates for high-dose chemotherapy.

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Materials and methods

Patient eligibility

The study was reviewed and approved by the participating institutional ethics committees (J. nr. H-KA-99040-GMS), and all patients gave written informed consent before study entry. The trial was performed before clinical trials had to be registered at http://clinicaltrials.gov/ but has been subsequently registered as NCT01016795. Patients were eligible if they were candidates for high-dose chemotherapy and auto-SCT, were between 18 and 65 years of age and with histologically documented malignant lymphoma in relapse, refractory or with PR to initial induction therapy. Previous hematopoietic growth factor administration had to be completed at least 1 week before study entry. Patients were required to have an ANC of $\geqslant 1.5 \times 10^9$/L, a plt count of $\geqslant 100 \times 10^9$/L, serum creatinine $<150\,\mu$mol and bilirubin, aspartate aminotransferase, and alanine aminotransferase less than twice the upper limit defined at the investigating laboratory. In addition, patients had to have an ECOG (Eastern Cooperative Oncology Group) performance status 0, 1 or 2, and life expectancy of >6 months with treatment.

Patients were excluded if they had received DexaBEAM or miniBEAM[27] therapy or previous high-dose chemotherapy with autologous progenitor cell support. Because of the possibility of systemic allergic-like reactions, patients with severe allergic history (seasonal/recurrent asthma, anaphylactic-type events, angioedema/recurrent urticaria and allergy to insect venoms) were not included. Other exclusion criteria included clinical and/or microbiological signs of infection or fever, HIV seropositivity, known allergy to *Escherichia coli*-derived products or significant nonmalignant disease. A history of asthma or other significant IgE-mediated hypersensitivities or required concurrent use of β-adrenergic blocking agents was prohibited because of potential interactions with the SCF premedication.

Hematopoietic growth factors

Both filgrastim and SCF were supplied by Amgen Inc. (Thousand Oaks, CA, USA). SCF was expressed in *E. coli* as a 166 amino acid nonglycosylated protein and included methionine at the N-terminus (r-metHuSCF); this was provided as either an aqueous solution or a lyophilized powder. Both the SCF and filgrastim were kept refrigerated at 2–8 °C until the time of injection. Lyophilized SCF was reconstituted with sterile water for injection before s.c. administration.

Hematopoietic progenitor cell enumeration

All progenitor cell analyses were performed at an experienced stem cell laboratory using standardized full-blood $CD34^+$ methodology.[8,27–29] Samples were analyzed by flow cytometry on a FACScan (Becton Dickinson, Mountain View, CA, USA) or a comparable cytometer after labelling with phycoerythrin (PE)-conjugated anti-CD34 (HPCA-2; Becton Dickinson) of a total of minimum 100 000 nucleated $CD45^+$ leukocytes. Cells stained with HPCA-2 were identified by FL2 and low side scatter, and background labelling was subtracted.

Central analysis of leukapheresis samples

Available leukapheresis samples were shipped to one laboratory and analyzed by flow cytometry to identify and enumerate abnormal lymphocyte subsets, including B-cell light-chain restriction.

Samples ($N=41$) from 21 (66%) of the 32 patients were analyzed on a four-color FACSCalibur (Becton-Dickinson) with a six-tube panel of 14 pre-titrated monoclonal antibodies directed against 12 Ags: CD56 FITC, CD33 PE, CD3 PerCP and APC, CD19 APC, CD45 FITC and APC, CD34 PE, κ and λ light chains, CD20 PerCP, CD4 FITC, TCR gamma, δPE, and CD8 PerCP. Two tubes contained unstained cells and IgG1 Ab controls for unspecific staining, respectively.

The frozen samples were thawed in a 37 °C water bath and washed once in a PBS solution containing DNAase (100 mL Buffer A, 0.5 mL $MgCl_2$ 500 mM + 2.5 mL DNAase (Pulmozyme 1 mg/mL)) to prevent clumping and cell loss caused by sticky DNA fragments from dead cells, and then washed once in buffer A (500 mL PBS, 2.5 g BSA + 10 mL Na-EDTA stock solution pH 5.5–5.7) and adjusted to a cell concentration of 2×10^7 cells per mL.

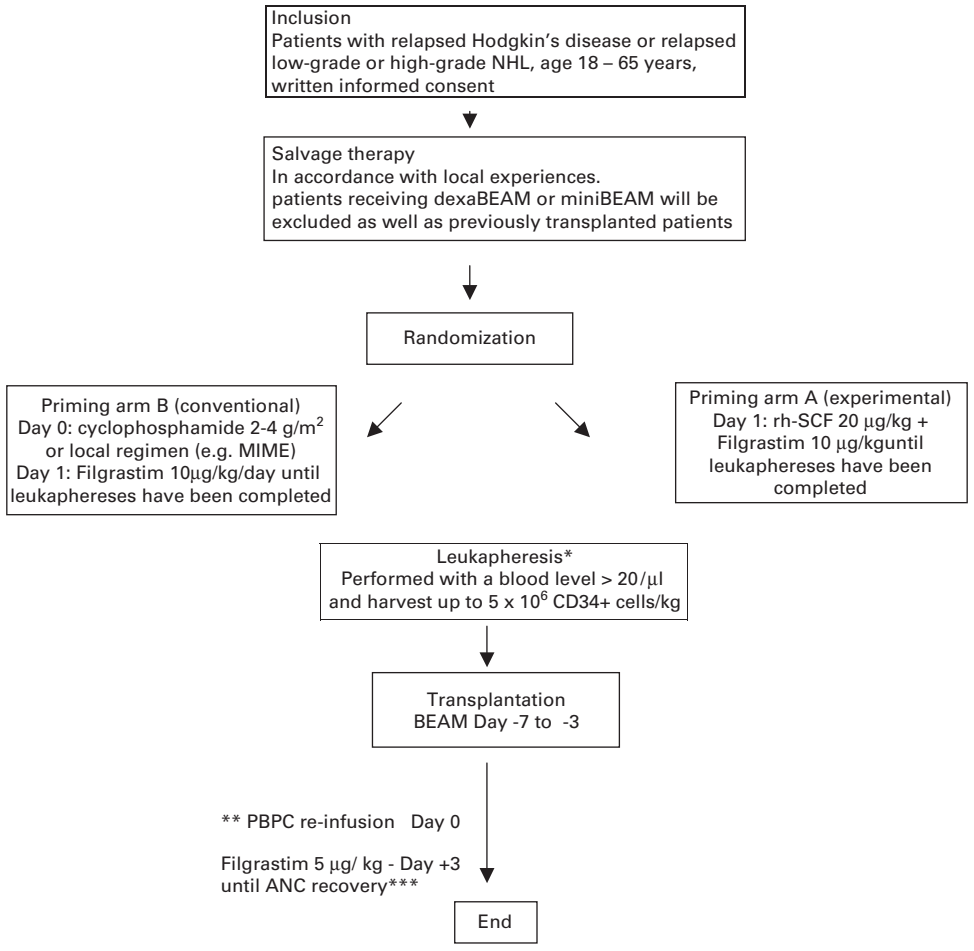
Samples of 50 μL were mixed with 50 μL Ab solutions and incubated for 30 min at 4 °C, then washed twice in standard buffer, resuspended to a volume of 3–400 μL and analyzed immediately. In each tube, 100 000 events (500 000 events in the κ–λ tube) were acquired ungated and later analyzed in CellQuestPro (BD Biosciences, San Jose, CA, USA) using a FSC–SSC live gate, excluding debris and dead cells. Data analysis was then repeated including dead cells as a control measure. Reported data are on live gated cells. Calculations were performed in Excel based on exported statistics files.

Study design

This was a randomized, open-label, multicenter study. It consisted of a priming phase, a collection phase, a transplantation phase and a 90-day follow-up. An overview of the treatment of patients for this study is shown in Figure 1. Enrolment was offered to all eligible patients presenting at the participating institutions.

Priming phase. Patients were randomized in a 1:1 ratio to either chemotherapy combined with 10 μg/kg/day filgrastim (control arm B), administered by s.c. injection for 14 days, or the combination of 10 μg/kg/day filgrastim and SCF administered s.c. at a dose of 20 μg/kg/day (experimental arm A) for 8 days. Different injection sites were used for each cytokine. The details of the cytokine dosing and leukapheresis procedures for each treatment group are shown in Figure 1.

The first dose of SCF was administered on an in-patient basis, with overnight observation after the injection. Subsequent doses were administered on an outpatient basis with at least 4 h of observation. All patients treated with SCF received prophylaxis against mast cell-mediated adverse effects. Prophylaxis consisted of 50 mg diphenhydramine orally every 6 h, 150 mg ranitidine orally every



* Leukapheresis will start when CD34+ count in peripheral blood ≥ 20/µl and will be performed daily until an optimal yield of 5 x 10^6 CD34+ cells/kg is achieved (see section 6.2 for details) or until 5 apheresis are performed.

** The total yield of CD34+ cells to be transfused will be 5 x10^6 CD34+ cells/kg

*** Leukocyte count >1 x 10^9/l each day for three days or > 10 x 10^9/l for one day.

Figure 1 Study design and treatment flow charts.

12 h, two puffs of albuterol metered-dose inhaler and 120 mg pseudoephedrine by sustained release. Administration of diphenhydramine and ranitidine began 12–24 h before the first dose of SCF and was timed so that a dose of all four medications was delivered approximately 1 h before each injection of SCF. Diphenhydramine and ranitidine therapy was continued until 48 h after the last SCF injection.

Collection phase. Leukaphereses were performed using a Baxter Fenwall CS3000 (Baxter, Deerfield, IL, USA), a Cobe Spectra (COBE Laboratories, Lakewood, CO, USA) or a comparable machine. A blood volume of approximately 10 L was processed at each daily leukapheresis. Patients were scheduled to undergo leukapheresis on the day when the $CD34^+$ cell blood level reached $\geqslant$20/uL.

The required minimum cumulative yield of $CD34^+$ cells was 5.0×10^6 cells/kg actual body weight (bw), which represented an acceptable standard in the literature at the time this trial was initiated. If this minimum yield was not achieved after a total of at least 4 leukapheresis, the patient was classified as a mobilization failure (poor mobilizers) and was managed with either further leukapheresis or supplementation of the PBPCs with harvested BM for progenitor cell support. The leukapheresis product collected on each day was processed and cryopreserved. Patients with $< 5 \times 10^6$ $CD34^+$ cells/kg harvested proceeded to treatment phase at the discretion of the investigator. No assessment of $CD34^+$ cell subsets or malignant clone cells was performed on the leukapheresis product before storage.

Transplantation and follow-up phase. Within 6 weeks of completing the leukapheresis procedure, patients were admitted to hospital and treated with myeloablative therapy (BEAM) followed by autologous PBPC transplantation at day 0. BEAM conditioning therapy consists of BCNU 300 mg/m^2 i.v. (30 min) q.d. on day –7, etoposide

200 mg/m^2 i.v. (30 min) q.d. on days –7 to –4, Ara-C 200 mg/m^2 i.v. (30 min) b.i.d. on days minus –7 to –4 and melphalan 140 mg/m^2 i.v. (5 min) q.d. on day –3. After 2 days of rest, the total number of 5×10^6 CD34$^+$/kg PBPCs were reinfused on day 0.

Beginning on day 0 of transplantation, filgrastim was administered at a dose of 5 μg/kg/day from day 3 until ANC recovery (ANC $\geqslant 1.0 \times 10^9$/L for 3 consecutive days or $\geqslant 10 \times 10^9$/L for 1 day) or for a maximum of 28 days (whichever occurs first). Antibiotics, blood products, and i.v. fluids were administered as clinically indicated. Complete blood counts were obtained daily until ANC was $\geqslant 1.0 \times 10^9$/L and the plt level was $\geqslant 20 \times 10^9$/L and were obtained three times per week thereafter until the plt count was $\geqslant 50 \times 10^9$/L on two determinations separated by a minimum of 48 h. Patients were assessed daily during hospitalization and weekly after discharge until plt recovery had occurred.

Throughout the study, investigators were allowed to prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care (except additional cytokines, IFN, WBC transfusions, other investigational agents or β-adrenergic blocking agents). Concomitant medications prescribed for serious adverse events and disallowed concomitant medications were recorded on the case record form. Administration of chemotherapy (other than as specifically defined in the protocol) was not allowed while the subject was on study (that is, once enrolled, until the end of study).

Plt transfusions were given to maintain a plt count of $>10 \times 10^9$/L, and transfusion dates were recorded. A plt transfusion comprised either a defined number of units of random donor plts or a single-donor pack, depending on the practice of the site. If the subject was febrile or suffered from hemorrhage, plts could be given at the discretion of the investigator, and all dates were then recorded. An investigator comment on the case record form was required for all plt transfusions given when plt count was $>10 \times 10^9$/L.

All patients were discharged from the hospital when the ANC was $\geqslant 1.0 \times 10^9$/L and i.v. antibiotic therapy was no longer necessary. Subjects underwent an end of treatment phase evaluation at 30 days after receiving the last dose of filgrastim or when their plt count exceeded 50×10^9/L, whichever occurred first, after late intensification therapy. At the end visit, a physical examination, including ECOG status, engraftment and disease status, was performed.

Adverse events were recorded in each phase of the study. Special attention was paid to any allergic-type reactions. The relationship to the investigational drug was scored.

Objectives, clinical end point, sample sizes and data analysis
The clinical objective of this randomized phase II trial was to compare the safety and toxicity (primary), as well as efficacy (secondary), of a growth factor 'only' priming by r-metHuSCF plus filgrastim (arm A) with that of chemotherapy plus filgrastim priming (arm B).

Primary end points. Safety and toxicity were assessed by morbidity, including unexpected adverse events associated with the priming and the transplantation phases occurring during the study, and measured and graded by CTC (common toxicity criteria). Other assessments included days with neutropenia and thrombocytopenia, days with fever on antibiotics, days in hospital and numbers of transfusions.

Secondary end points. Efficacy was assessed by graft-related variables during the mobilization, collection and follow-up phase. These included the blood level of CD34$^+$ cells from day 3 to the end of priming and harvest, total number of CD34$^+$ cells harvested per day of leukapheresis and time to three-lineage engraftment by a fixed autografting of 5.0×10^6 cells/kg bw.

Sample size considerations. Sample size considerations were based on feasibility and estimates from published toxicity data,[14–26] as well as a retrospective toxicity evaluation of a standard chemotherapy-based priming procedure.[11] A sample size of 60 was chosen to gain as much information as possible about the response variables defined. The study was closed by Amgen after inclusion of 32 patients, which however was considered sufficient by the investigators to evaluate the trial end points.

Statistical analysis. All subjects who were randomized and had growth factor administered during the priming phase of the trial were analyzed on intent to treat.

Statistical analysis was used to determine whether there were any significant differences between the groups with respect to the described end points above. An exact binomial test was used to test for equality of proportions between the two treatment arms.

Results

Study population
Demographic-, disease- and therapy-related data from the 32 subjects who were randomized and entered into the study, with 16 patients in each arm, is given in Table 1. Of these, 29 (91%) patients entered the collection phase, and 25 (78%) reached the target of 5 million CD34$^+$ cells/kg. A total number of 21 (67%) patients completed the transplantation phase before study closure.

Safety evaluation summarized by adverse events
During the mobilization phase we identified five patients with eight serious events, four in each arm. Two patients (13%) in arm A suffered from one allergic, two febrile and one rigor episode. Three patients (19%) in arm B suffered from two febrile and one hematological episodes of severe grading (Table 2). Evaluation during the transplantation phase identified three patients with four serious events, one hepatic failure in arm A and central nervous system affection, seizure and skin erythema in arm B (results not shown).

A summary of all adverse events revealed 30 (94%) patients suffering from 132 events of all grading reported in accordance with the CTC criteria. As expected, 10 patients (63%) reported erythema, pigmentation or reaction from

Table 1 Demographic, disease and therapy characteristics at the time of inclusion

Variable	*Arm A: r-metHuSCF+filgrastim mobilization number (%)*	*Arm B: chemotherapy+filgrastim mobilization number (%)*
Gender male/female	13 (81%)/3 (19%)	8 (50%)/8 (50%)
Age years median (range)	50 (20–68)	50 (23–63)
ECOG performance scale 0/1	11 (69%)/4 (25%)	11 (69%)/4 (25%)
Disease MHdg/NHL low/high grade	3 (19%)/4 (25%)/9 (56%)	4 (25%)/1 (6%)/11 (69%)
Marrow involvement +/−	2 (13%)/11 (69%)	4 (25%)/11 (69%)
Disease status	0 (0%)/4 (25%)/10 (63%)	3 (19%)/4 (25%)/7 (44%)
Prim Refract/PR/relapse		
Previous radiotherapy	3 (19%)	4 (25%)
Ann Arbor staging I–II/III/IV	6 (38%)/3 (19%)/7 (44%)	3 (19%)/5 (31%)/8 (50%)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; NHL = non-Hodgkin's lymphoma; r-metHuSCF = recombinant methionyl human SCF.

Table 2 Serious adverse events during mobilization phase

Variable	*Arm A: r-metHuSCF+ filgrastim mobilization number*	*Arm B: chemotherapy+filgrastim mobilization number*
Number of patients	16	16
Total number of events:	4	4
Allergic reactions	1	0
Fever	2	2
Rigors	1	0
Hematological	0	1
Respiratory	0	1

Abbreviation: r-metHuSCF = recombinant methionyl human SCF.

Table 3 Number of leukapheresis to achieve 5 million $CD34^+$ cells/kg

Variable	*Arm A: r-metHuSCF+ filgrastim mobilization number*	*Arm B: chemotherapy+ filgrastim mobilization number*
Number of patients	16	16
Number of daily leukapheresis 1/2/3/4/5/>5	1/7/0/0/1/5	10/2/0/0/1/2
Leukapheresis not triggered	2	1

Abbreviation: r-metHuSCF = recombinant methionyl human SCF.

the injection site in arm A, which was not observed in arm B. On the contrary 9 (56%) from arm B had fever, compared with only 3 (19%) from arm A. A detailed analysis review of data revealed no differences when the two arms were compared for adverse events from central or peripheral nervous system (dizziness, headache and seizure), gastrointestinal tract (diarrhea, nausea, pain and vomiting), heart (rate and rhythm), hematopoietic system (penia and hemorrhage), liver, musculoskeletal, urinary or respiratory systems and the skin (data not shown).

Toxicity evaluation

In arm B, neutropenia and thrombocytopenia were documented in 14 (88%; median 4 days, range 0–10) and in 3 (19%; median 0 days, range 0–6) patients, respectively. In comparison, no cytopenia was documented in arm A. Fever occurred in 8 (50%) patients from arm B, and in only 1 (6%) patient from arm A. Transfusions were administered 12 times in arm B, but never in arm A.

Efficacy evaluation

The mobilization of $CD34^+$ cells reached a maximum on day 5 of 30 $CD34^+$/mikrol (median, range 5–162) in arm A compared with a maximum on day 10 of 124/mikrol (median, range 2–206) in arm B.

The median (range) number of $CD34^+ \times 10^6$ cells/kg harvested was 5.4 (1.9–8.5) in arm A and 8.3 (1.4–16.1) in arm B. Only 1/14 (7%) patient in arm A compared with 10/15 (67%) patients in arm B reached the target of 5×10^6 $CD34^+$/kg on the first day of leukapheresis ($P = 0.00018$). The number of leukapheresis required to achieve the target of 5.0 million $CD34^+$ cells/kg varied from 1 to 5 days. In arm A 9/14 (56%) patients reached the target when compared with 13/15 (81%) patients in arm B ($P = 0.24$; Table 3).

Post transplant, after reinfusion of the exact number of 5 million $CD34^+$ cells/kg bw, there was no difference between the two groups in terms of the number of days in hospital, number of days on antibiotics, number of transfusions (results not given) or number of serious adverse events by CTC criteria (results not given). Of special interest, the time to reticulocyte, plt and netrophilocyte recoveries were median (range) 12 days (10–14), 11 days (8–16) and 11 days (10–12) in arm A, and 12 days (9–16), 11 days (8–16) and 10 days (9–13) in arm B, respectively.

Graft evaluation

Enumeration of selected subsets of interest in the harvested leukapheresis products was performed by flow cytometry, and it confirmed the higher level of $CD34^+$ cells harvested in arm B compared with arm A (Table 4). However, no difference in B- and T-cell levels were identified and no differences in the level of potential clonal cells were found (Table 5).

Discussion

In this randomized pilot study the addition of r-metHuSCF (ancestim) to filgrastim was compared with conventional

Table 4 Leukocyte subsets in leukapheresis samples analyzed by flow cytometry

Treatment group	*% CD34+*	*% CD33+*	*% CD19+*	*% CD56+ CD3−*	*% CD3+*	*% CD4+ CD3+*	*% CD8+ CD3+*	*% TCR+ CD8+ CD3+*
Arm A (N = 10) r-metHuSCF and filgrastim mobilization								
Mean	1.4	65.0	5.7	12.3	23.2	7.2	17.5	2.8
Median	0.7	65.6	0.5	10.6	21.8	6.8	17.8	2.3
Min	0.1	46.5	0.0	2.4	11.5	1.8	7.1	0.2
Max	4.4	84.4	33.5	25.2	48.7	18.9	28.0	8.2
N	10	10	10	10	10	10	10	10
Arm B (N = 11) chemotherapy and filgrastim mobilization								
Mean	3.1	69.5	3.6	9.3	21.2	8.4	13.8	1.5
Median	2.5	77.4	0.8	9.8	14.1	6.4	8.2	0.7
Min	0.3	37.9	0.0	3.5	3.2	1.8	2.0	0.1
Max	8.2	95.6	27.0	13.8	46.4	22.2	26.9	4.0
N	11	11	11	11	11	11	11	11

Abbreviation: r-metHuSCF = recombinant methionyl human SCF.

Table 5 Normal and clonal B cells in leukapheresis samples analyzed by flow cytometry

Treatment group	*Diagnosis*	*Patients*	*Clonal B cells*	*Live gate events *10^5*	*% CD19$^+$ in live gate*	*<100 CD19$^+$ events*	*% CD19$^+$ <1*		*% CD19$^+$ 1–10*		*% CD19$^+$ >10*	
		N	N	*Mean*	*Mean*	N	N	*Mean*	N	*Mean*	N	*Mean*
Arm A ($N = 10$) r-metHuSCF and filgrastim mobilization	B-NHL	5	0	2.8	6.9	3	4	0.0	0		1	34.1
	HD	2	0	2.2	10.2	0	0		1	4.9	1	15.6
	T-NHL	3	0	2.3	1.8	0	2	0.4	1	4.6	0	
Arm B ($N = 11$) chemotherapy and filgrastim mobilization	B-NHL	7	2	1.7	5.2	1	3	0.3	3	2.7	1	27.6
	HD	2	0	2.0	0.5	0	2	0.5	0		0	
	T-NHL	2	0	1.9	1.1	0	1	0.1	1	2.0	0	

Abbreviations: B-NHL = B-cell non-Hodgkin's lymphoma; HD = Hodgkin's disease; r-metHuSCF = recombinant methionyl human SCF; T-NHL = T-cell non-Hodgkin's lymphoma.

chemotherapy plus filgrastim priming. It was the study hypothesis that removing chemotherapy and adding rhSCF to the priming scheme would allow a sufficient PBPC harvest in most patients safely and with reduced toxicity and side effects. The clinical trial was designed to generate data on safety, toxicity and efficacy during the experimental nonchemotherapy-based priming regimen (arm A) and compare with a conventional chemotherapy-based strategy (arm B) in relapsed or refractory lymphoma patients who were considered candidates for high-dose therapy.

Our study shows that removal of chemotherapy from the mobilization regimen will reduce toxicity. However, the drawback is a reduced ability to harvest adequate CD34$^+$ cell numbers.

Administration of the experimental combination of SCF and G-CSF was safe and well tolerated with a low risk of *adverse events* consistent with previous observations.[14–26] No serious allergic-like reactions were observed and the absence of such events in the present study might be related to the careful screening for allergy history, systematic premedication or relatively small patient numbers for detecting low-frequency events. In other large randomized studies, such severe events were reported in 3–10% of patients.[18,25]

The *efficacy* of the priming regimens was compared by the number of leukapheresis required to achieve a target of 5 million CD34$^+$ cells/kg bw, which varied from 1 to 5 days. In the experimental arm, 9/14 (56%) patients reached the target, compared with 13/15 (81%) in the conventional arm. This result supported the observation that the maximum blood level and harvest of CD34$^+$ cells in the chemotherapy arm was increased 5–6 and 1.5 times, respectively (data not shown). Finally, only 1 (6%) patient compared with 10 (63%) patients in arm B reached the target of 5 million CD34$^+$ cells/kg bw on the first day of leukapheresis. These results are not in line with those observed in other trials of the combination of SCF and G-CSF to improve PBPC collection.[18–26,30–33] The explanation might be that the present experimental arm was compared with standard chemotherapy plus G-CSF and included patients with malignant lymphoma, most of whom were heavily pre-treated before relapse or disease progression and even included patients with primary refractory disease.

In summary, a whole spectrum of priming studies has evaluated the effect of SCF on the grade of mobilization. However, one missing link in the literature is the present comparison between experimental combined growth factors and conventional chemotherapy priming. The present pilot study is the first to evaluate this comparison and conclude that a phase III trial is unlikely to change the present recommendation for malignant lymphoma patients

considered candidates for high-dose therapy that conventional chemotherapy plus G-CSF is the most effective priming regimen with an acceptable toxicity and safety profile.

Conflict of interest

The authors declare no conflict of interest.

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